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ORIGINAL ARTICLE

Beckwith–Wiedemann syndrome: Clinical, histopathological and molecular study of two Tunisian patients and review of literature

Hela Sassi[1,2] | **Yasmina Elaribi**[1,2] | **Houweyda Jilani**[1,2] | **Imen Rejeb**[1] | **Syrine Hizem**[1,2] | **Molka Sebai**[1,2] | **Nadia Kasdallah**[2,3] | **Habib Bouthour**[2,4] | **Samia Hannachi**[2,5] | **Jasmin Beygo**[6] | **Ali Saad**[7,8] | **Karin Buiting**[6] | **Dorra H'mida Ben-Brahim**[7,8] | **Lamia BenJemaa**[1,2]

[1]Department of Congenital and Hereditary Diseases, Mongi Slim Hospital Marsa, Tunis, Tunisia

[2]Faculty of Medicine of Tunis, University Tunis El Manar, Tunis, Tunisia

[3]Paediatric Department, Military Hospital of Tunis, Tunis, Tunisia

[4]Department of Paediatric Surgery, Tunis, Tunisia

[5]Laboratory of Pathology Anatomy and Cytology, Tunis, Tunisia

[6]Institute for Human Genetics, Essen University Hospital, Essen, Germany

[7]Department of Cytogenetics and Reproductive Biology, Farhat Hached University Hospital, Sousse, Tunisia

[8]Faculty of Medicine of Sousse, University of Sousse, Sousse, Tunisia

Correspondence
Hela Sassi, Department of Congenital and Hereditary Diseases, Mongi Slim Hospital Marsa, Tunis, Tunisia.
Email: hella.sassi@gmail.com

Abstract

Background: Beckwith–Wiedemann syndrome (BWS) is a rare overgrowth syndrome characterized by congenital malformations and predisposition to embryonic tumors. Loss of methylation of imprinting center 2 (IC2) is the most frequent alteration and rarely associated with tumors compared to paternal uniparental disomy of chromosome 11 (UPD(11)pat) and gain of methylation of imprinting center 1.

Methods: Our study aimed to describe the clinical, histopathological and genetic characteristics of two patients and establish genotype-phenotype correlations. The clinical diagnosis was based on the criteria defined by the international expert consensus of BWS. Molecular study of 11p15.5 methylation status was assessed using methylation-specific-multiplex ligation probe amplification (MS-MLPA).

Results: Patients were aged 12 months and 3 months and fulfilled the clinical score of BWS. MS-MLPA showed molecular alterations consisting of loss of methylation in IC2 (IC2-LOM) at the maternal allele for one patient and a mosaic UPD(11)pat for the second patient in whom follow-up at 6months revealed adrenocortical carcinoma (ACC) with low grade of malignancy. Molecular subtypes guide the follow-up and tumor surveillance, our major concern.

Conclusion: We have to take into account the psychological impact of a possible tumor whatever the underlying mechanism is. Nevertheless, the tumor risk remains high for UPD(11)pat. Our study extended the phenotype of BWS with absence of macrosomia in Tunisian patients, contrasting with literature, and added a supplementary case of ACC in the tumor spectrum of BWS patients with UPD(11)pat.

Keywords
adrenocortical tumors, Beckwith–Wiedemann syndrome, correlation, epigenetic, genomic imprinting

1 | INTRODUCTION

Beckwith–Wiedemann syndrome (BWS; OMIM #130650), first described in 1963 (Beckwith, 1963; Wiedemann, 1964), is a constellation of clinical manifestations which may include macrosomia, macroglossia, abdominal wall defects, neonatal hypoglycemia, excessive lateralized growth and predisposition to embryonic tumors (Engström et al., 1988; Thorburn et al., 1970). Multiple epigenetic and/or molecular genetic mechanisms have been described, resulting in the deregulation of the imprinted genes of the 11p15 region: *H19* (*103280) and *IGF2* (*147470) in the telomeric domain, and *CDKN1C* (*600856), *KCNQ1* (*607542) and *KCNQ1OT1* (*604115) genes in the centromeric domain (Hatada et al., 1996; Henry et al., 1991; Reik et al., 1995; Weksberg et al., 2003). These genes are involved in cell cycle progression and growth control and regulated by two independent imprinting centers (IC1/IC2). The most frequent mechanism is a loss of IC2 methylation on the maternal allele accounting for about 50% of BWS cases (Brioude, Kalish, et al., 2018). The international consensus of 2018 established a clinical score with cardinal and suggestive features and introduced a new terminology "Beckwith–Wiedemann spectrum" (Brioude, Kalish, et al., 2018). In the Tunisian population, the tumoral and genetic spectrum of BWS remains not well known. To our best knowledge, only one Tunisian study was published on confirmed BWS with partial loss of methylation in imprinting center 2 in a 45-day-old girl having a benign adrenocortical tumor (H'mida Ben-Brahim et al., 2015). In our study, we aim to report the clinical, histopathological and genetic profile of two Tunisian patients with a confirmed BWS and discuss genotype-phenotype correlation.

2 | METHODS

2.1 | Patients

We conducted a retrospective study, between January 2018 and December 2020, including patients referred to the department of Congenital and Hereditary Diseases at Mongi Slim Hospital Marsa of Tunis, for polymalformative syndrome suggestive of BWS. We collected all the clinical data related to this syndrome and made an extensive genetic survey for each patient. The World Health Organisation charts were used to interpret growth parameters. (https://www.who.int/tools/child-growth-standards/standards). The clinical diagnosis was based on criteria defined by the specific clinical score of BWS established by international expert consensus in 2018 (Brioude, Kalish, et al., 2018). The clinical follow-up, at Mongi Slim Hospital Marsa of Tunis, was also adapted according to the experts' recommendations (Brioude, Kalish, et al., 2018).

2.2 | Histological and immunohistochemistry study

Histological samples of the left adrenal gland were analyzed. A macroscopic analysis was carried out on the postoperative specimen tissue, fixed in a 4% formalin solution. After formalin fixation, the fragments were dehydrated through different alcohols and then the alcohols were removed with xylene. After impregnation of the tissues with paraffin and rehydration, routine sections (3 μm) were stained with standard haematoxylin and eosin (HE). Immunohistochemistry study using a panel of antibodies was performed on formalin-fixed-paraffin-embedded sections (Table S1). After revealing antigenic sites, endogenous peroxidase activity was blocked. The studied antibodies were revealed by the chromogen diaminobenzidine (DAB). Slides were counterstained with HE. The pediatric score used to classify adrenocortical tumors was the Wieneke score (Wieneke et al., 2003).

2.3 | Genetic study

R-banding karyotype on lymphocytes was first performed. Genomic DNA was extracted from leukocytes using standard proteinase-K extraction protocol (Miller et al., 1988). The BWS epigenetic alterations in 11p15 region (IC1 and IC2), were studied with the SALSA MS-MLPA Probemix specific kit (ME030-C3 BWS/RSS; MRC Holland, Amsterdam, Netherlands) according to the manufacturer's protocol. Copy number analysis of 11p15 region (*H19* (NR_002196.2), *IGF2* (NM_000612.5; NM_001127598.2), *CDKN1C* (NM_000076.2), *KCNQ1* (NM_000218.2) and *KCNQ1OT1* (NR_002728.3)) was assessed by standardized ratios of the fluorescence signal generated by the amplification of the specific probes before digestion with

HhaI enzyme, using the ranges validated by this kit. Comparison of the peaks after digestion allowed the study of the methylation status in 11p15 region.

2.4 | Literature review

A PubMed search using the keywords "Beckwith–Wiedemann syndrome", "Beckwith–Wiedemann expert consensus," imparted articles of interest that were selected considering the number of patients included, the confirmation of the molecular mechanisms with particular selection of the cohorts with cancers.

3 | RESULTS

3.1 | Clinical reports

Two Tunisian patients suspected of BWS, from unrelated phenotypically normal young parents (mean age at conception: 30 years), from spontaneous pregnancy, were involved. The family history was negative for both patients.

3.2 | Patient 1 (P1)

The first patient was a 12-month-old girl. The antenatal follow-up revealed an omphalocele of 3 cm long axis. She was born at 36th gestational week by cesarean section with good adaptation to external life. Measurements at birth were between 50 and 85 percentiles for weight (3100 g), between 90 and 97 percentiles for height (50 cm) and between 3rd and 15th percentiles for head circumference (32.5 cm). The examination at birth found macroglossia and omphalocele, without neonatal hypoglycemia. She underwent surgery for the omphalocele with simple postoperative follow-up. Psychomotor development was normal.

At genetic consultation, she had average weight and head circumference, height at +1.8 SD with left excessive lateralized growth (Figure 1.I.a). She had dysmorphic features (Figure 1.I.a-f). Cardiovascular and neurological examinations were normal. There was no visceromegaly. Skin examination revealed facial naevus simplex on the forehead, two plane centimetric angiomas on the thorax and neck.

Trans-fontanellar, cardiac and abdominal ultrasounds did not find abnormalities. Laboratory tests showed peripheral hypothyroidism and normal alpha-fetoprotein (AFP) level (12.73 ng/mL).

3.3 | Patient 2 (P2)

The second patient was a 3-month-old boy. The antenatal ultrasonographic examination showed umbilical hernia. He was born by vaginal delivery at 37th gestational week. He had normal measurements at birth for weight (3180g, 50 percentiles); head circumference (34cm, 50 percentiles) and had height of 46cm (3–15 percentiles). Birth examination revealed an isolated uncomplicated

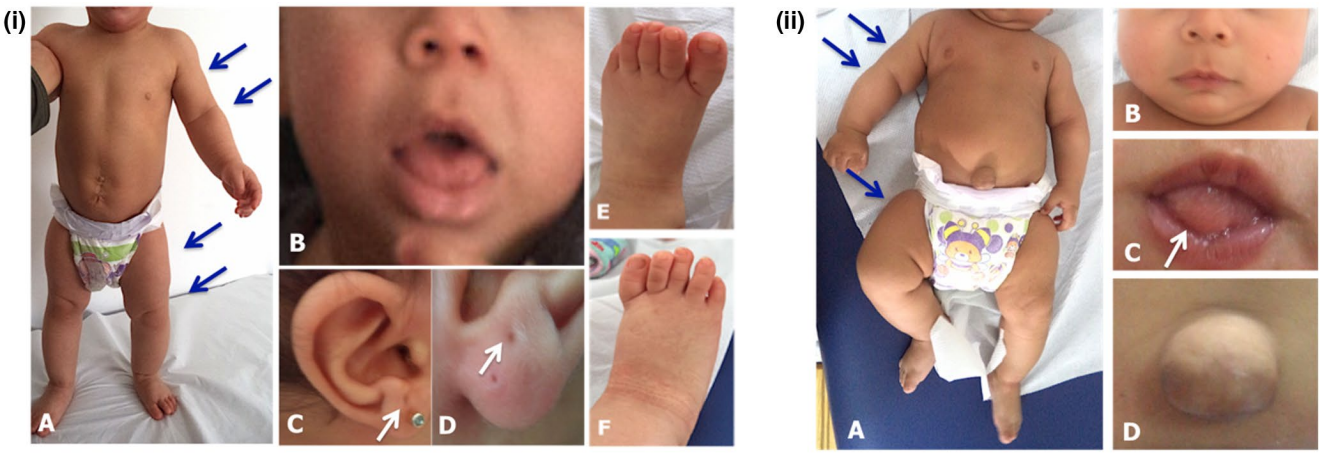

FIGURE 1 Phenotype of patients. Patient 1: (a) The blue arrows show the discreet left lateralized growth. The neck was short. (b) Facial dysmorphology: thin eyebrows, mid-face hypoplasia, depressed nasal root, anteverted nostrils, short columella, long philtrum, thin upper lip, thick everted lower lip and macroglossia. (c,d) Bilateral ear pits highlighted with the arrows. (e,f) She had clinodactyly of the 5th toes, a low implantation of the right big toe, and overlapping of the 2nd and 3rd right toes. Patient 2: (a) The blue arrows show the discreet right lateralized growth. (b) Facial dysmorphic features: thin eyebrows, long eyelashes, depressed nasal root, bulbous nose, anteverted nostrils, short columella, long philtrum, thin lips and micrognathism. (c) The arrow shows hypertrophy of the right hemi tongue. (d) Umbilical hernia measuring 2.5 cm long axis

umbilical hernia. At genetic consultation, he had normal anthropometric parameters with right lateralized body overgrowth (Figure 1.II.a). Mild dysmorphic features were noted (Figure 1.II.a-d). Cardiac and abdominal ultrasounds were normal. During the clinical follow-up, at the age of 6 months, P2 had an acute abdominal syndrome related to a heterogeneous and finely calcified mass in the left adrenal gland, suggestive of neuroblastoma (Figure 2). He was operated with simple postoperative follow-up.

3.4 | Histological and immunohistochemistry results

In patient 2, gross examination of the surgical specimen of the left adrenal gland showed an encapsulated nodule of firm consistency, weighing 20 g and measuring 5x4x3 cm, with focal necrosis, suspected of malignancy (Figure 3).

Histological staining showed tumor proliferation surrounded by a fibrous capsule of variable thickness related to partial capsular invasion (Figure 4a-b). The tumor was arranged in cords and nests (40% of tumor surface) with some trabecular and alveolar areas and foci of acellular fibrosis (Figure 4c). Cellular density was moderate to marked. Tumor cells, round medium-sized, had granular eosinophilic cytoplasm (Figure 4d-e). The nuclei had moderate atypia with focal presence of marked anysokaryosis. The mitotic count was estimated at 25 mitoses/20 high power fields (HPF; Figure 4f). Foci of confluent tumor necrosis were estimated at 20% of the tumor surface with the presence of focal calcifications (Figure 4g). There was

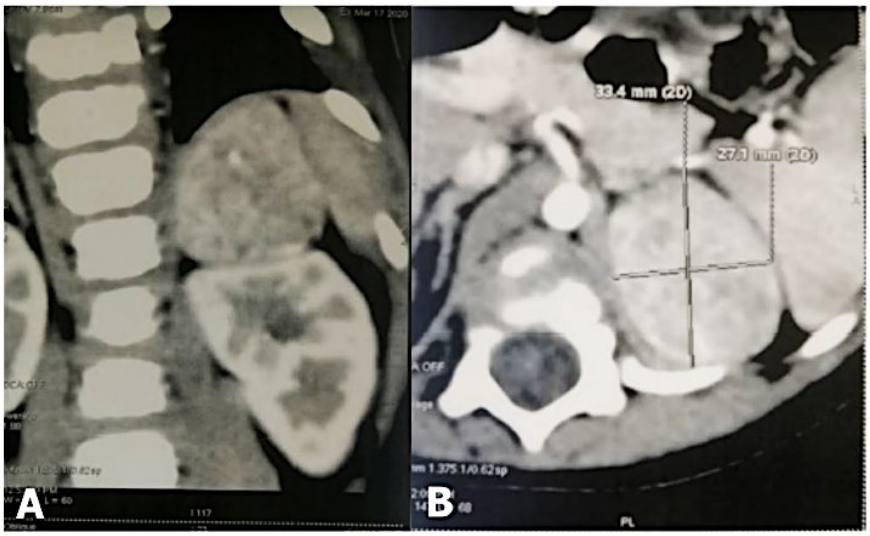

FIGURE 2 Computed tomography scan in patient 2. (A,B). Heterogeneous and finely calcified process of 3.5 cm long axis in left adrenal gland

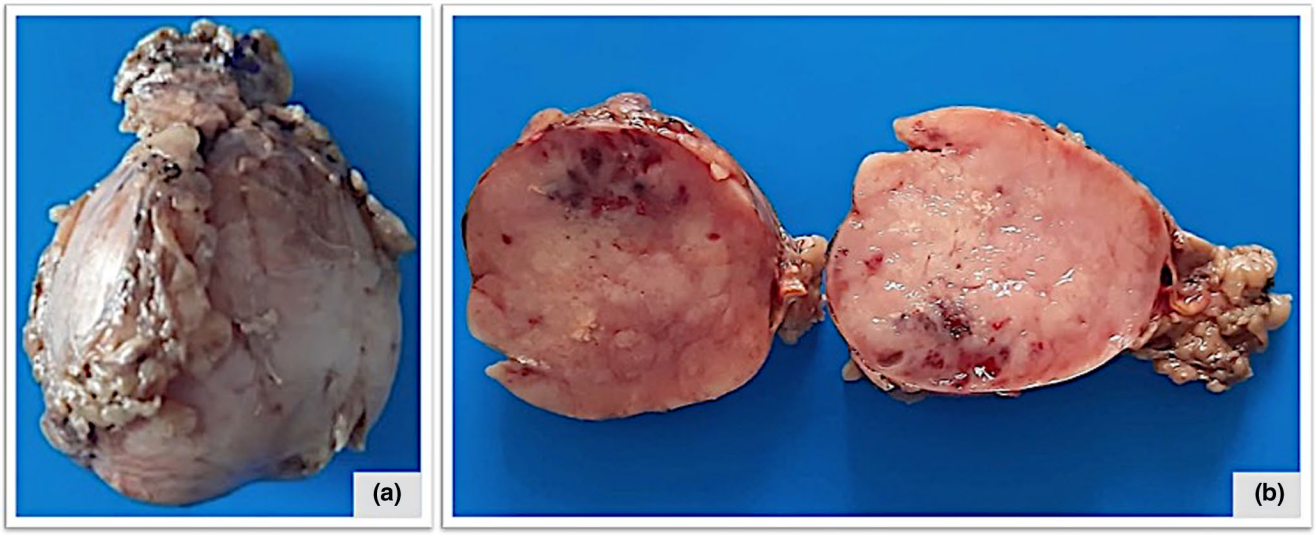

FIGURE 3 Macroscopic study of left adrenal gland process in patient P2, (a) Fixed tissue. (b): Cross section showing micro-nodular solid appearance of the process with hemorrhagic and necrotic alterations

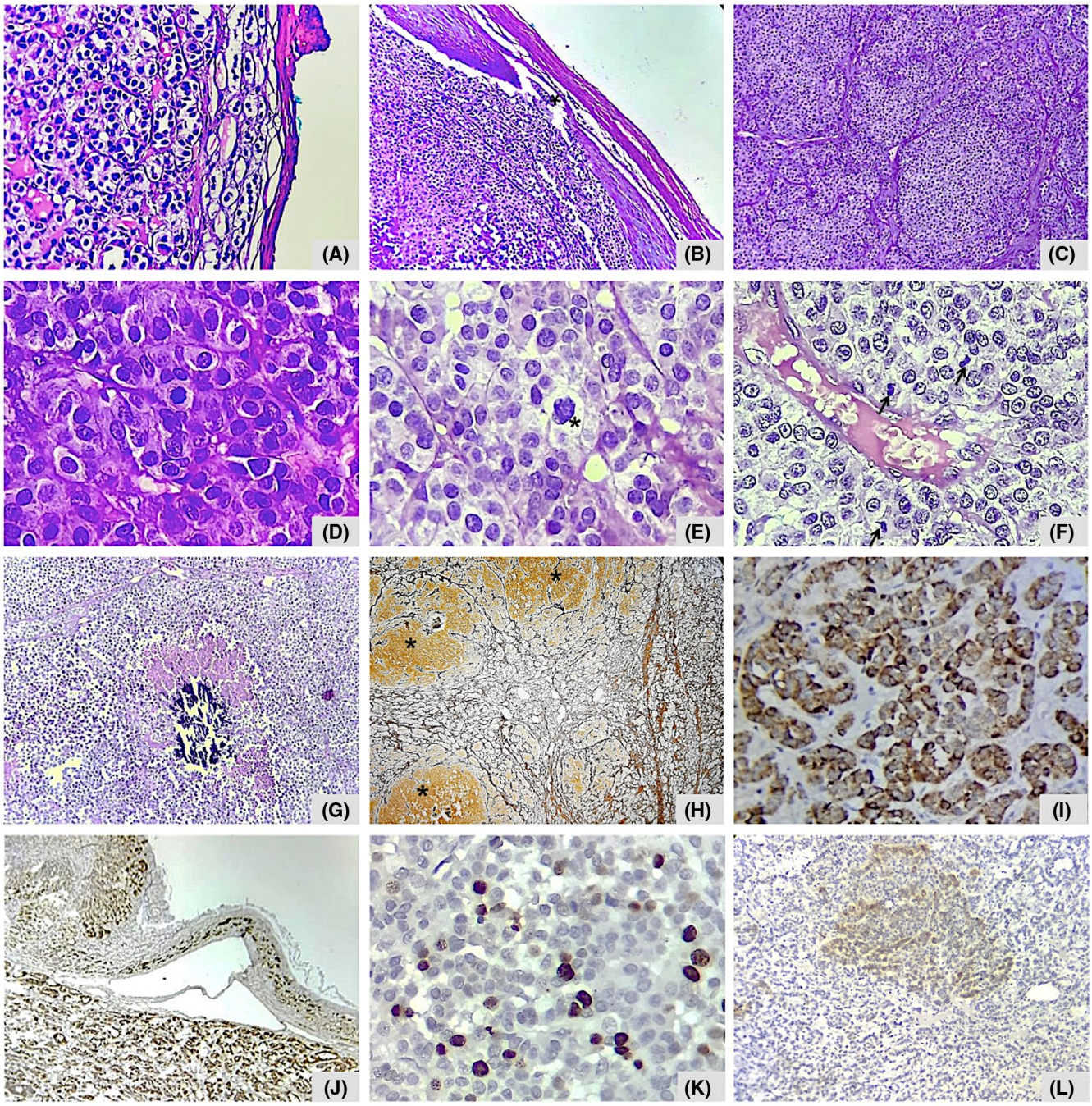

FIGURE 4 Histological and immunohistochemistry results in patient P2. (A) Encapsulated tumor; (B) Capsule focally and partially invaded (*); (C) Micro-nodular morphology; (D) Round tumor cells (x400); (E): Cellular atypia (*); (F): Mitosis (→); (G): Tumor necrosis calcified in the center; (H) Special staining of reticulin: disorganized reticulinic network within solid territories (*); (I) Cytoplasmic staining with anti-Melan A antibody; (J) Nuclear staining with anti-beta-catenin antibody; (K): Nuclear staining with anti-Ki67 antibody; (L): Cytoplasmic staining with anti-inhibin antibody

neither pericapsular fat invasion nor tumor vascular emboli. The residual adrenal gland had normal morphology.

The special reticulin staining showed a disorganized reticulin network in the solid territories (Figure 4h). In the immunohistochemical study (Figure 4i-l, Table S1), the tumor cells were positive for anti-Melan A and anti-Beta-Catenin antibodies (Figure 4i,j). Index proliferation Ki67 was evaluated at 20% (Figure 4k).

The results of pathological examination and immunohistochemical study concluded to left adrenocortical tumor with low grade of malignancy (Wienecke score: 3) whose surgical excision was complete.

3.5 | Genetic investigation

Both patients had normal chromosomal formula. MS-MLPA showed normal copy number in 11p15.5 region and confirmed the diagnosis of BWS by loss of methylation in IC2 (IC2-LOM) at the maternal allele for P1 and a mosaic paternal uniparental disomy of chromosome 11 [UPD(11)pat] for P2. MS-MLPA on parents' blood DNA of the two families was normal.

4 | DISCUSSION

4.1 | Clinical study

This work represents a descriptive study of two Tunisian patients fulfilling the clinical score of BWS that has been molecularly confirmed.

Data on antenatal ultrasounds in BWS have concluded to orientation signs mainly umbilical hernia (60%), omphalocele (50%–80%), renal hypertrophy (65%) and hydramnios (50%–60%; Galerneau, 2018). P1 and P2 had prenatally diagnosed omphalocele and umbilical hernia, respectively. In the recent and large European cohort of Barisic et al., the mean gestational age was comparable in boys and girls born alive 36.4 ± 3.4 amenorrhea week with prematurity (<37 amenorrhea week) of 37% (Barisic et al., 2018). Spontaneous prematurity has been described in P1 girl. The mean maternal and paternal age was respectively 29.6 ± 5.4 and 32.7 ± 6.4 years, in concordance to our data, and only 27% of fathers were under 30 years (Barisic et al., 2018). The advanced paternal age is known to induce *de novo* mutations and epi-genetic modifications, particularly abnormalities of the parental imprint in the spermatogonia. Studies indicate that age-related alteration in sperm DNA methylation in elder men can affect early embryonic development (Simon et al., 2014). In our study, the parents were young at the time of conception.

The diagnosis of BWS was suggested at 12 months in P1 and at 3 months in P2. Barisic et al. (2018) suspected BWS in 39.9% cases before birth, 36.3% at birth, 7.6% in first week of life and 16.2% in the first year of life. Duffy et al. (2019) had concluded that diagnostic confirmation was made in prenatal (9.4%), neonatal (45.3%) and beyond 28 days (45.3%), without any significant difference between ethnic groups (p: .377), which is consistent with our patients, where the diagnosis of BWS was suspected after 28 days of life.

The mean birth weight was 4006 ± 754 g for boys and 3766 ± 747 g for girls (Barisic et al., 2018). In our study, our patients did not have macrosomia.

The type and frequency of major congenital anomalies related to BWS in our patients are shown in Table 1 compared to the data available in literature. In cardinal features, macroglossia, omphalocele and excessive lateralized growth were predominant, in agreement with our patients. The main suggestive features were macrosomia, facial naevus simplex and ear pits. The latter two signs were not constant in our patients (Table 1).

4.2 | Genetic study

The 11p15 region comprises genes organized in clusters, distributed in two functionally independent domains, regulated by 2 imprinting centers (IC1/IC2). *H19* and *IGF2* in the telomeric domain, and *CDKN1C*, *KCNQ1* and *KCNQ1OT1* genes in the centromeric domain are controlled by IC1 and IC2 respectively. Differential methylation of these two ICs is responsible for maternal expression of the *H19*, *KCNQ1* and *CDKN1C* genes and paternal expression of the *IGF2* and *KCNQ1OT1* genes (Brioude, Kalish, et al., 2018; Choufani et al., 2010).

DNA methylation abnormalities are the most involved mechanisms, the most frequent of which (~50%) is the loss of methylation at the IC2, as is the case in patient 1 (Brioude, Kalish, et al., 2018; Choufani et al., 2010; Eggermann et al., 2014). The other mechanisms are estimated as follows: segmental UPD(11)pat (20%) observed in P2, gain of methylation at maternal allele in IC1 (IC1-GOM; 5%–10%), *CDKN1C* mutations in 5% of sporadic cases and 40% of familial cases and chromosomal rearrangements (deletion, duplication) within chromosome 11p15 (<5%; Brioude, Kalish, et al., 2018).

4.3 | (Epi)genotype-phenotype correlations in Beckwith–Wiedemann syndrome

There is a correlation between (epi)genotype and phenotype, hence the importance of determining the molecular mechanism in BWS. We compared the phenotype of our patients to that described in large cohorts (Brioude et al., 2013; Ibrahim et al., 2014; Maas et al., 2016; Mussa, Molinatto, et al., 2016; Mussa, Russo, et al., 2016; Table 2). IC2-LOM is characterized by prematurity (41.3%), neonatal and/or postnatal macrosomia (52%–58%), facial naevus simplex (50%–75%), auricular abnormalities (50%–75%), macroglossia (70%–97%), umbilical hernia (55%–67%) and omphalocele (30%–91%; Brioude et al., 2013; Ibrahim et al., 2014; Maas et al., 2016; Mussa, Molinatto, et al., 2016; Mussa, Russo, et al., 2016; Table 2). While UPD(11)pat is characterized by neonatal macrosomia (64%–87%), macroglossia (69%–86%), excessive lateralized growth (57%–85%), organomegaly (38%–58%), absence of abdominal

TABLE 1 Frequency of major and minor abnormalities in Beckwith–Wiedemann syndrome in our patients and review of literature

	Our study	Correa et al. (2020)	Wang, Xiao, et al. (2020)	Duffy et al. (2019)	Barisic et al. (2018)	Bilgin et al. (2018)	H'mida Ben-Brahim et al. (2015)	Mussa et al. (2013)	Moreno-Salgado et al. (2013)
Population (n=) (%)	2	8	31 (%)	139 (%)	234 (%)	28 (%)	1	46 (%)	19 (%)
Country	Tunisia	India	China	Review[a]	Europe	Turkey	Tunisia	Italy	Mexico
Prematurity	1/2	ND	ND	62 (44.2)	37	10 (35.7)	0	ND	5/18 (33.3)
Cardinal features									
Macroglossia	1/2	8/8	18 (58.1)	101 (72.7)	189 (80.8)	21 (75.0)	0	40 (86.9)	17 (89)
Omphalocele	1/2	5/8	3 (9.7)	29 (20.9)	122 (52.1)	12 (42.8)	0	5 (10.9)	6 (31.5)
Excessive Lateralized growth	2/2	3/8	9 (29.0)	115 (82.5)	49 (20.9)	21 (75.0)	0	30 (65.2)	7 (37)
Multifocal and/or bilateral WilmsTumor or nephroblastomatosis	0/2	0/8	0	ND	ND	4 (14.2)	0	4 (8.7)	0
Hyperinsulinism	0/2	0/8	1 (3.2)	54 (38.6)	ND	ND	0	ND	ND
A/P/Placenta[b]	0/2	0/8	ND	ND	A: 6 (2.5)/P: 9 (3.8)	A: 2 (7.1)	0	A+P: 4 (8.7)	A: 1 (5.3)
Suggestive features									
Macrosomia	0/2	2/8	10 (32.3)	91 (65.2)	120 (37)	9 (32.1)	1	33 (71.7)	8/17 (47)
Facial naevus simplex	1/2	1/8	11 (35.5)	69 (49.3)	ND	13 (46.4)	0	22 (47.8)	9 (47.4)
Hydramnios and/or placentomegaly	0/2	0/8	5 (16.1)	35 (25.4)/25 (17.7)	ND	8 (28.5)/1 (3.5)	ND	ND	5/17 (29.4)
Ear creases and/or pits	1/2	7/8	14 (45.2)	98 (70.4)	ND	11 (39.2)	0	14 (30.4)	10 (52.6)
Transient hypoglycaemia	0/2	3/8	5 (16.1)	90 (64.5)	ND	10 (35.7)	0	10 (21.7)	11/17 (61.1)
Typical BWS tumour spectrum[c]	1/2	0/8	0	33 (23.4)	ND	5 (17.8)	0	2 (4.3)	0
Nephromegaly and/or hepatomegaly	0/2	1/8	12 (38.7)	38 (26.9)/34 (24.6)	63 (26.9)/39 (16.7)	17 (60.7)	0	29 (63.1)	10 (52.6)
Umbilical hernia and/or diastasis recti	1/2	5/8	23 (74.2)	53 (37.7)/26 (18.4)	45 (19.2)/13 (5.6)	12 (42.8)	0	13 (28.3)/11 (23.9)	6 (31.5)/1 (5.2)

(Continues)

TABLE 1 (Continued)

	Our study	Arroyo Carrera et al. (1999)	Weng et al. (1995)	Elliott et al. (1994)	Martínez et al. (1992)	Engström et al. (1988)	Pettenati et al. (1986)
Population (n=) (%)	2	18 (%)	15 (%)	76 (%)	39 (%)	388 (%)	22 (%)
Country	Tunisia	Spain	USA	United Kingdom	Mexico	Review	USA
Prematurity	1/2	6 (33.3)	8 (53)	65 (85.5)	ND	ND	7 (33)
Cardinal features							
Macroglossia	1/2	18 (100)	13/14 (93)	74 (97)	37 (94.4)	(82)	22 (100)
Omphalocele	1/2	10 (55.6)	10 (66)	34 (44.7)	16 (41)	ND	8/22 (36.4)
Excessive lateralized growth	2/2	ND	7 (47)	18 (24)	8 (20)	ND	4/18 (22.2)
Multifocal and/or bilateral WilmsTumor or nephroblastomatosis	0/2	ND	0	1 (1.3)	ND	ND	1/20 (5)
Hyperinsulinism	0/2	ND	ND	ND	ND	ND	ND
A/P/Placenta[a]	0/2	ND	ND	P: 6 (8)	ND	ND	ND
Suggestive features							
Macrosomia	0/2	ND	ND	67 (88)	ND	(38.5)	ND
Facial naevus simplex	1/2	ND	8/14 (57)	47 (62)	ND	(32.1)	13/19 (68.4)
Hydramnios and/or placentomegaly	0/2	ND	5/6 (83)/9/10 (90)	25 (33)/ND	ND	ND	5/17 (29.4)/ND
Ear creases and/or pits	1/2	ND	8/14 (57)	58 (76)	ND	(38.0)	15/20 (75)
Transient hypoglycaemia	0/2	ND	5/12 (42)	48 (63)	ND	(30.4)	10/20 (50)
Typical BWS tumour spectrum[c]	1/2	ND	0	2 (2,6)	ND	ND	0
Nephromegaly and/or hepatomegaly	0/2	5 (27.8)/3 (16.7)	10/14 (71)	45 (59)/23 (25)	ND	(23)/(32.1)	15/15 (100)/17/18 (94.4)
Umbilical hernia and/or diastasis recti	1/2	4 (22.2)	4 (27)/4 (27)	24 (31.6)/3 (4)	18 (46.2)/	(75.2)	11/22 (50)/4/22 (18.2)

Abbreviation: BWS, Beckwith–Wiedemann syndrome; ND, not determined.

[a]9 studies (Brioude et al., 2013; DeBaun et al., 2002; Ibrahim et al., 2014; Lin et al., 2016; Luk et al., 2017; Maas et al., 2016; Mussa, Molinatto, et al., 2016; Mussa, Russo, et al., 2016; Sasaki et al.,2007; Weksberg et al.,2001).

[b]Adrenal cortex cytomegaly (A); pancreatic adenomatosis (P); placental mesenchymal dysplasia.

[c]Neuroblastoma, rhabdomyosarcoma, unilateral Wilms tumour, hepatoblastoma, adrenocortical carcinoma, phaeochromocytoma.

TABLE 2 Significant (epi)genotype-phenotype correlations ($p < .05$) in large correlation studies in Beckwith–Wiedemann syndrome (Brioude et al., 2013; Ibrahim et al., 2014; Maas et al., 2016; Mussa, Russo, et al., 2016)

Clinical features	IC1-GOM	UPD(11)pat	IC2-LOM	*CDKN1C* mutation	Study and *p*	P1/P2
Prematurity	28.6%	18.1%	41.3%	62.5%	Mussa et al. ***p* < .05**	+/−
Hydramnios	3.8%	24.4%	71.8%	ND	Ibrahim et al. $p > .05$	−/−
	35.5%	14.9%	15.3%	0%	Mussa et al. ***p* < .05**	
Neonatal macrosomia	96.8%	64.4%	58.4%	40%	Mussa et al. ***p* < .05**	−/−
	73.3%	87.5%	51.8%	ND	Maas et al. ***p* < .05**	
Postnatal macrosomia	29.7%	8.2%	62.1%	ND	Ibrahim et al. $p > .05$	−/−
	45.2%	39.1%	56.3%	60%	Mussa et al. ***p* < .05**	
Normal growth	0%	24.1%	21.1%	40%	Mussa et al. ***p* < .05**	+/+
Excessive lateralized growth	40%	81%	20.3%	3.1%	Brioude et al. ***p* < .05**	+/+
	7.6%	57.3%	35.1%	ND	Ibrahim et al. ***p* < .05**	
	45.2%	82.8%	45.8%	0%	Mussa et al. ***p* < .05**	
	57.9%	85.7%	33%	ND	Maas et al. ***p* < .05**	
Macroglossia	85.7%	86.2%	97.6%	93.9%	Brioude et al. ***p* < .05**	+/−
	8.1%	22.5%	69.4%	ND	Ibrahim et al. ***p* < .05**	
	90.3%	69%	88.4%	70%	Mussa et al. ***p* < .05**	
	85%	79.1%	86.2%	ND	Maas et al. $p > .05$	
Organomegaly	64.5%	58.3%	39.1%	19.2%	Brioude et al. ***p* < .05**	−/−
	16.5%	38.3%	45.1%	ND	Ibrahim et al. ***p* < .05**	
	67.7%	36.8%	27.9%	10%	Mussa et al. ***p* < .05**	
	35%	32%	24%	ND	Maas et al. $p > .05$	
Omphalocele	10%	13.2%	66.7%	71%	Brioude et al. ***p* < .05**	+/−
	1.7%	6.9%	91.3%	ND	Ibrahim et al. ***p* < .05**	
	9.7%	6.9%	30%	70%	Mussa et al. ***p* < .05**	
	0%	12.8%	32%	ND	Maas et al. ***p* < .05**	
Umbilical hernia	28.6%	48.7%	67.1%	93.9%	Brioude et al. ***p* < .05**	−/+
	10.8%	33.8%	55.4%	ND	Ibrahim et al. ***p* < .05**	
	9.7%	18.4%	13.2%	0%	Mussa et al. $p > .05$	
	40%	42.1%	43.9%	ND	Maas et al. $p > .05$	
Diastasis recti	23.8%	33.3%	42.9%	ND	Ibrahim et al. ***p* < .05**	**−/−**
	48.4%	23%	23.7%	0%	Mussa et al. ***p* < .05**	
	33.3%	23.5%	19.4%	ND	Maas et al. $p > .05$	
No abdominal defect	29%	51.7%	33.2%	30%	Mussa et al. ***p* < .05**	**−/−**
Facial naevus simplex	11.1%	29.7%	57%	24.1%	Brioude et al. ***p* < .05**	+/−
	3.7%	21.1%	75.3%	ND	Ibrahim et al. ***p* < .05**	
	22.6%	34.5%	48.4%	50%	Mussa et al. ***p* < .05**	
	15%	35.9%	53.4%	ND	Maas et al. ***p* < .05**	
Ear creases and/or pits	27.3%	50%	65.4%	90.9%	Brioude et al. ***p* < .05**	+/−
	6.8%	17.9%	75.3%	ND	Ibrahim et al. ***p* < .05**	
	22.6%	39.1%	50.5%	60%	Mussa et al. ***p* < .05**	
	16%	60%	57%	ND	Maas et al. $p > .05$	
Renal abnormalities	32.3%	26.4%	8.9%	20%	Mussa et al. ***p* < .05**	**−/−**
	40%	44.7%	13.2%	ND	Maas et al. ***p* < .05**	

(Continues)

TABLE 2 (Continued)

Clinical features	IC1-GOM	UPD(11)pat	IC2-LOM	*CDKN1C* mutation	Study and *p*	P1/P2
Urethral abnormalities	22.6%	6.9%	4.2%	10%	Mussa et al. ***p* < .05**	−/−
Hypoglycemia	32.4%	60.5%	40.2%	37.5%	Brioude et al. ***p* < .05**	−/−
	8.5%	28.9%	62.7%	ND	Ibrahim et al. $p > .05$	
	35.5%	34.5%	31.6%	20%	Mussa et al. $p > .05$	
	46.2%	66.7%	62.9%	ND	Maas et al. $p > .05$	
Malignant tumors	28.6%	17.3%	3.1%	8.8%	Brioude et al. ***p* < .05**	−/+
	8.5%	6.7%	0.9%	ND	Ibrahim et al. ***p* < .05**	
	25.8%	14.9%	1.6%	0%	Mussa et al. ***p* < .05**	
	31.6%	13.6%	2.6%	ND	Maas et al. $p = (-)$	
Benign tumors	12.9%	6.9%	2.1%	0%	Mussa et al. ***p* < .05**	−/−

Note: Bold value indicates *p* value < .05.

CDKN1C (NM_000076.2).

Abbreviations: (−), absent; (+), present; IC1-GOM, gain of methylation in imprinting center 1; IC2-LOM, loss of methylation in imprinting center 2; ND, not determined; *p*, *p* value; UPD(11)pat, Paternal uniparental disomy of chromosome 11.

defect (51.7%; Brioude et al., 2013; Ibrahim et al., 2014; Maas et al., 2016; Mussa, Molinatto, et al., 2016; Mussa, Russo, et al., 2016; Table 2). The phenotypic particularity in our patients was the absence of macrosomia, contrasting with literature. In the Italian series of Mussa, Molinatto, et al. (2016), Mussa, Russo, et al., 2016, normal growth was reported in 21.1% of cases ($p < .05$; Table 2).

The risk of malignancy in BWS, independent of the molecular mechanism, is estimated between 5% and 15%, being higher at birth and reaching the general population before the onset of puberty (Brioude, Kalish, et al., 2018; Rump et al., 2005). The risk of malignant and benign tumors is about 1%–3% and 2.1% respectively in IC2-LOM. It is higher in IC1-GOM (8.5%–28%) and UPD(11)pat (6%–17%; Brioude et al., 2013; Ibrahim et al., 2014; Mussa, Molinatto, et al., 2016; Mussa, Russo, et al., 2016; Table 2).

In large worldwide cohorts (total: 2,256), where tumor type has been correlated with molecular subtypes, the following tumor types have been identified in UPD(11)pat (79/346): 31 Wilms tumors, 22 hepatoblastomas, 8 adrenocortical carcinomas, 5 neuroblastomas, 3 pheochromocytomas, 3 nephroblastomas, 2 leukemias, 1 ganglioneuroma, 1 hemangiotelioma, 1 myopepithelial cell carcinoma, 1 pancreatoblastoma, and 1 rhabdomyosarcoma (Alsultan et al., 2008; Bliek et al., 2004; Brioude et al., 2013; Cöktü et al., 2020; Eltan et al., 2020; Gaston et al., 2001; Hertel et al., 2003; H'mida Ben-Brahim et al., 2015; Ibrahim et al., 2014; Kim et al., 2019; Maas et al., 2016; Mussa, Molinatto, et al., 2016; Mussa, Russo, et al., 2016; Sasaki et al., 2007; Weksberg et al., 2001; Wijnen et al., 2012; Table 3). This underlines the great variability of tumor types in this molecular subtype.

Adrenocortical tumors were also reported in five studies by IC2-LOM with pauci-symptomatic presentation and described in UPD(11)pat in large studies where the phenotype was not well reported (Alsultan et al., 2008; Bliek et al., 2004; Brioude et al., 2013; Cöktü et al., 2020; Eltan et al., 2020; Gaston et al., 2001; Hertel et al., 2003; H'mida Ben-Brahim et al., 2015; Ibrahim et al., 2014; Kim et al., 2019; Maas et al., 2016; Mussa, Molinatto, et al., 2016; Mussa, Russo, et al., 2016; Sasaki et al., 2007; Weksberg et al., 2001; Wijnen et al., 2012; Table 3). Kim et al. described a patient with hemihypertrophy and macroglossia related to UPD(11)pat. At 9 months, he developed an adrenocortical tumor of uncertain malignant potential occurring in the heterotopic adrenal cortex of liver (Kim et al., 2019). The age at diagnosis of the adrenocortical tumor was similar in the study of Cöktü et al. (Cöktü et al., 2020). P2 with the UPD(11)pat had low-grade adrenocortical carcinoma but with an earlier onset.

Most methylation changes in BWS patients are present in a mosaic state. These patients are somatic mosaics having normally methylated cells and cells with a loss of methylation at the IC2/gain of methylation at the IC1 or a UPD(11)pat. As this mosaicism might be restricted to certain tissue types, this could explain the different severity of clinical signs between patients (Brioude, Kalish, et al., 2018; Wang et al., 2020; Wang, Xiao, et al., 2020).

These data highlight that the majority of patients did not exhibit complete phenotypic features of BWS, unlike our patients. Pathologists should suggest to look for BWS in all cases of apparently sporadic and isolated adrenocortical tumor in the paediatric population (Wijnen et al., 2012).

TABLE 3 Tumor type in loss of methylation in imprinting center 2 and paternal uniparental disomy [UPD(11)pat] in large worldwide cohorts and literature review of adrenocortical tumors in these molecular subtypes of Beckwith–Wiedemann syndrome.

Studies	Cohort	Tumors in UPD(11)pat	Tumor type in UPD(11)pat	Tumors in IC2-LOM	Tumor type in IC2-LOM
Weksberg et al. (2001)	125	6/21	H (1); W (5)	5/35	H (2); G (1); R (2)
Gaston et al. (2001)	97	6/11	GG (1); Ph (1); W (4)	1/45	T (1)
Hertel et al. (2003)	1	—	—	1/1	A (1)
Bliek et al. (2004)	66	9/13	A (1); H (1); L (1); N (1); Ph (1); W (4)	2/27	H (1); T (1)
Sasaki et al. (2007)	47	2/7	H (2)	1/15	R (1)
Alsultan et al. (2008)	1	—	—	1/1	A (1)
Wijnen et al. (2012)	2	—	—	2/2	A (2)
Brioude et al. (2013)	407	17/81	A (2); H (2); L (1); N (1); R (1); W (10)	8/257	H (2); M (1); N (2); R (1); S (1); T (1)
Ibrahim et al. (2014)	637	8/16	A (1); H (5); W (2)	3/288	H (1); R (1); W (1)
Mussa, Russo, et al. (2016)	318	13/87	A (1); H (5); Hg (1); N (2); P (1); W (3)	4/190	N (2); R (1); g (1)
H'mida Ben-Brahim et al. (2015)	1	—	—	1/1	Ab (1)
Maas et al. (2016)	229	6/44	H (1); My (1); Ph (1); W (3)	3/114	H (1); W (2)
Kim et al. (2019)	1	1/1	A (1)	—	—
Cöktü et al. (2020)	321	10/64	A (1); H (5); N (1); Np (3)	3/208	As (1); H (1); W (1)
Eltan et al. (2020)	1	—	—	1/1	A (1)
Our study	2	1/1	A (1)	—	—
Total	2,256	79/346	A (8); GG (1); H (22); Hg (1); L (2); My (1); N (5); Np (3); Ph (3); P (1); R (1); W (31)	36/1,185	A (5); Ab (1); As (1); H (8); M (1); N (4); G (1); g (1); R (6); S (1); T (3); W (4)

Abbreviations: (—), not applied; A, adrenocortical carcinoma; Ab, benign adrenocortical tumor; As, astrocytoma; g, germinoma; G, gonadoblastoma; GG, ganglioneuroma; H, hepatoblastoma; Hg, Hemangiotelioma; IC2-LOM, loss of methylation in imprinting center 2; L, leukemia; M, melanoma; My, Myopepithelial cell carcinoma; N, neuroblastoma; Np, nephroblastoma; P, Pancreatoblastoma; Ph, pheochromocytoma; R, rhabdomyosarcoma; S, sarcoma; T, thyroid carcinoma; UPD(11)pat, Paternal uniparental disomy of chromosome 11; W, Wilms Tumor.

4.4 | Tumor surveillance

The aim was to improve the survival of these patients and reduce morbidity through early detection of tumors. Different parameters are taken into account, such as median age at tumor onset, tumor doubling time indicating the monitoring interval, the histological type, the tumor grade, surgical resection and the molecular subtype in BWS (Brioude, Kalish, et al., 2018; Maas et al., 2016; Table S2).

The excessive lateralized growth, described in our patients, and nephromegaly have been correlated with a higher risk of developing tumor in BWS, but without statistically significant difference (Maas et al., 2016).

In IC2-LOM, overall tumor risk is very low (2.6%) with the particularity of early onset (11 months) of Wilms tumors. Contrary to UPD(11)pat which risk is intermediate between IC2-LOM and IC1-GOM (Brioude, Hennekam, et al., 2018; Brioude, Kalish, et al., 2018; Maas et al., 2016). Thus, the BWS international consensus group suggested that abdominal ultrasound and AFP measurements are appropriate for the most at-risk molecular subgroups of BWS which are IC1-GOM and UPD(11)pat but did not recommend it in IC2-LOM (Brioude, Hennekam, et al., 2018; Brioude, Kalish, et al., 2018). The American Association for Research in Cancer (AACR) adopted a risk threshold of 1% for tumor surveillance and therefore recommends tumor screening for all cases of BWS spectrum, given the family psychological impact and the anticipatory anxiety of a new tumor (Brioude, Kalish, et al., 2018; Kalish et al., 2017). The decision of tumor monitoring can thus be discussed in multidisciplinary concertation meetings, particularly the case of P2, where surgical excision was considered complete with a low grade of malignancy not indicating adjuvant treatment. Regular monitoring has been proposed (Brioude, Kalish, et al., 2018).

4.5 | Genetic counselling and prenatal diagnosis

Various molecular mechanisms are associated with different risks of recurrence and prognoses. For our patients, the risk of recurrence is low (<1%; Brioude, Kalish, et al., 2018). For the subsequent pregnancies, we proposed a meticulous ultrasound follow-up, the detection of maternal serum increase in AFP in the second trimester and amniocentesis for fetal karyotype and MS-MLPA within 11p15 region, in case of suggestive ultrasound signs (Eggermann et al., 2016; Wang, Kupa, et al., 2020; Wang, Xiao, et al., 2020). The prenatal diagnosis of BWS is difficult owing to the mosaïcism and the risk of contamination by maternal cells (Brioude, Kalish, et al., 2018; Wang, Kupa, et al., 2020; Wang, Xiao, et al., 2020).

5 | CONCLUSIONS

Regardless of the molecular mechanism, we insist on the close follow-up of patients with BWS. We have shown that the phenotype in BWS was extended with the absence of macrosomia in both patients and added a well-documented case of low-grade adrenocortical carcinoma in the tumor spectrum in a BWS patient with UPD(11)pat. We have to consider BWS in case of embryonic tumors and in apparently isolated adrenocortical tumors in the pediatric population. The international databases listing phenotypic data and molecular mechanisms concerning BWS remain necessary given some exceptional and uncommon cases and to raise further awareness for BWS to enhance early diagnosis and tumor surveillance.

ETHICS COMPLIANCE

This study was approved by the local ethics committee of Mongi Slim Hospital. Parents of the probands signed a consent for genetic studies and publication of the medical information. No animal study was done in this work.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Guarantor of integrity of the entire study: Hela Sassi (MD), Yasmina Elaribi (MD), Houweyda Jilani (MD), Lamia BenJemaa (MD). Study concepts and design: Hela Sassi (MD), Yasmina Elaribi (MD), Houweyda Jilani (MD). Literature research: Hela Sassi (MD). Clinical studies: Hela Sassi (MD), Yasmina Elaribi (MD), Houweyda Jilani (MD), Imen Rejeb (PhD), Syrine Hizem (MD), Molka Sebai (MD), Nadia Kasdallah (MD), Habib Bouthour (MD). Experimental studies: Samia Hannachi (MD), Dorra H'mida Ben-Brahim (MD), Ali Saad (MD), Jasmin Beygo (PhD), Karin Buiting (PhD). Data analysis: Hela Sassi (MD). Statistical analysis: N/A (not apply). Manuscript preparation: Hela Sassi (MD). Manuscript editing: Hela Sassi (MD), Yasmina Elaribi (MD), Houweyda Jilani (MD), Dorra H'mida Ben-Brahim (MD), Jasmin Beygo (PhD), Karin Buiting (PhD). All authors read and approved the final version of this manuscript as submitted.

ORCID

Hela Sassi https://orcid.org/0000-0002-8999-929X
Jasmin Beygo https://orcid.org/0000-0002-6238-0382

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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